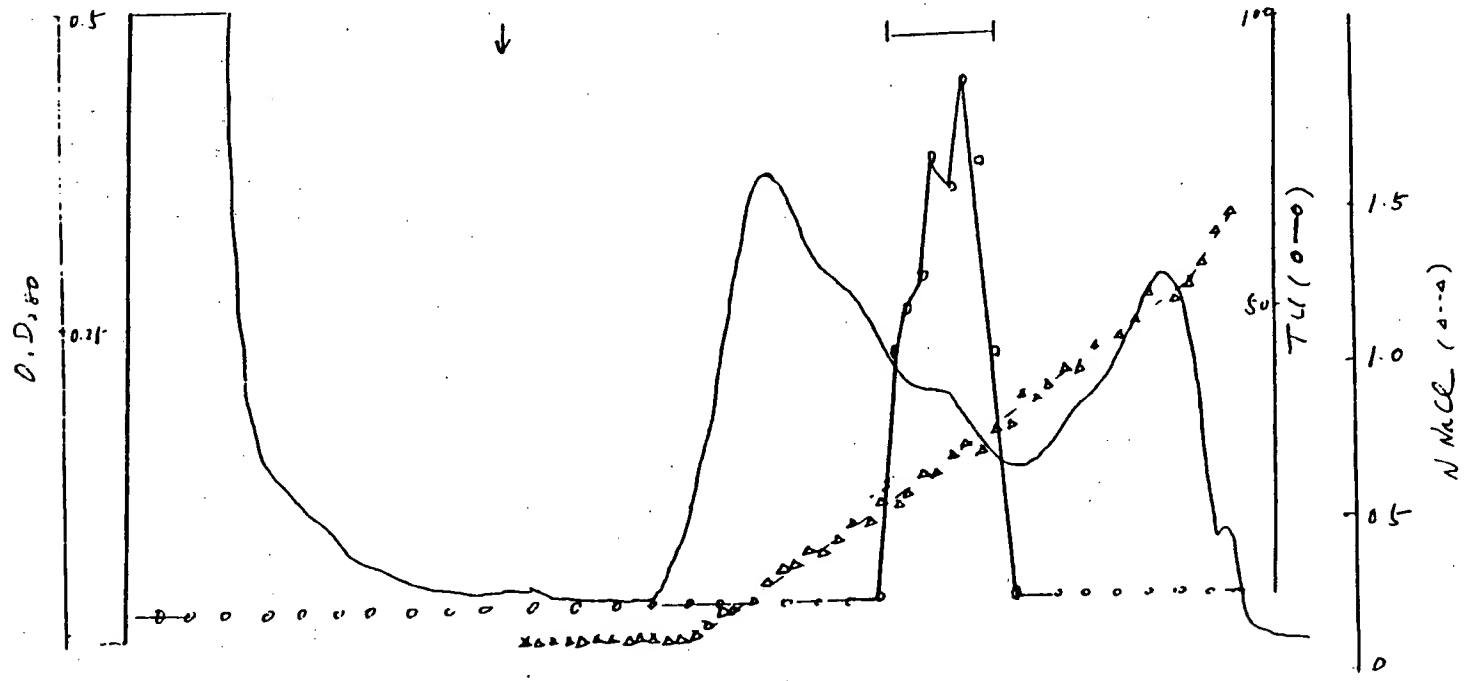
Fig. 1 Heparin Sepharose Chromatography



— O.D280

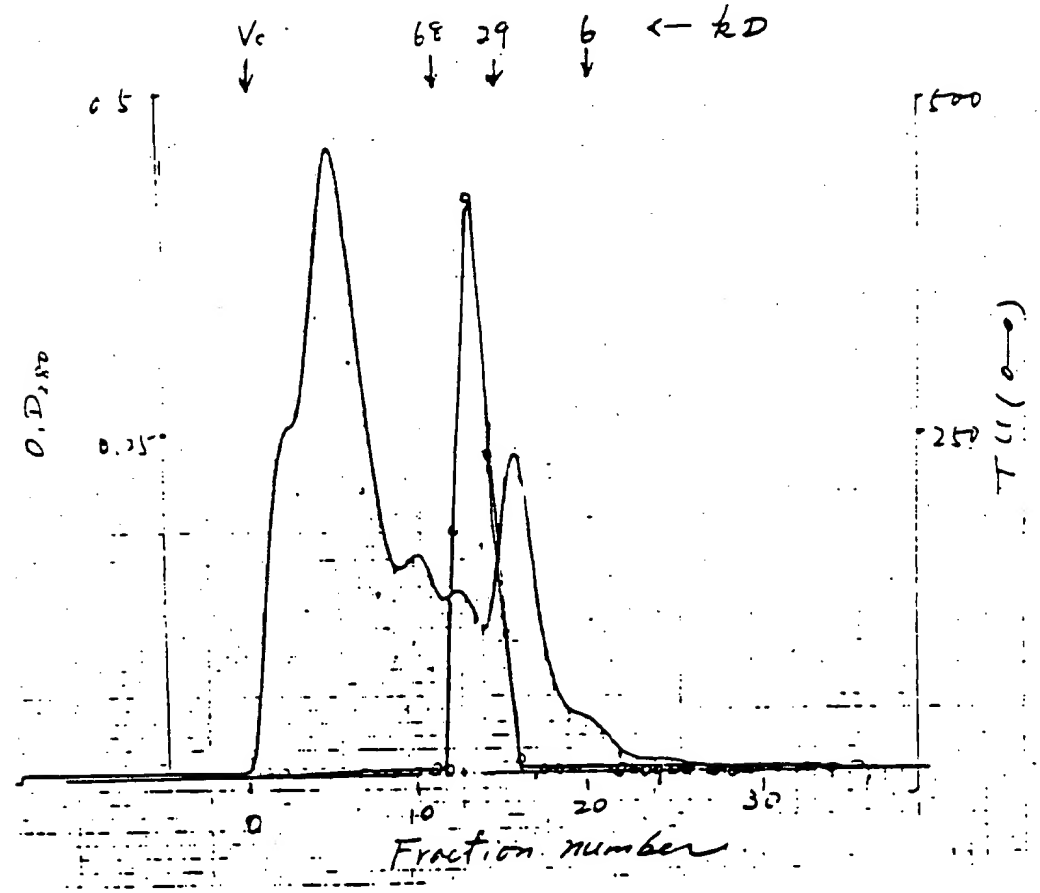
↓ gradient start

Δ--Δ conductivity

o—o GDNF activity in TU

|—| Fractions pooled for further analysis

Fig 2 FPLC Superose chromatography



— O.D280

o—o GDNF activity in TU

Fig. 3. RP-HPLC

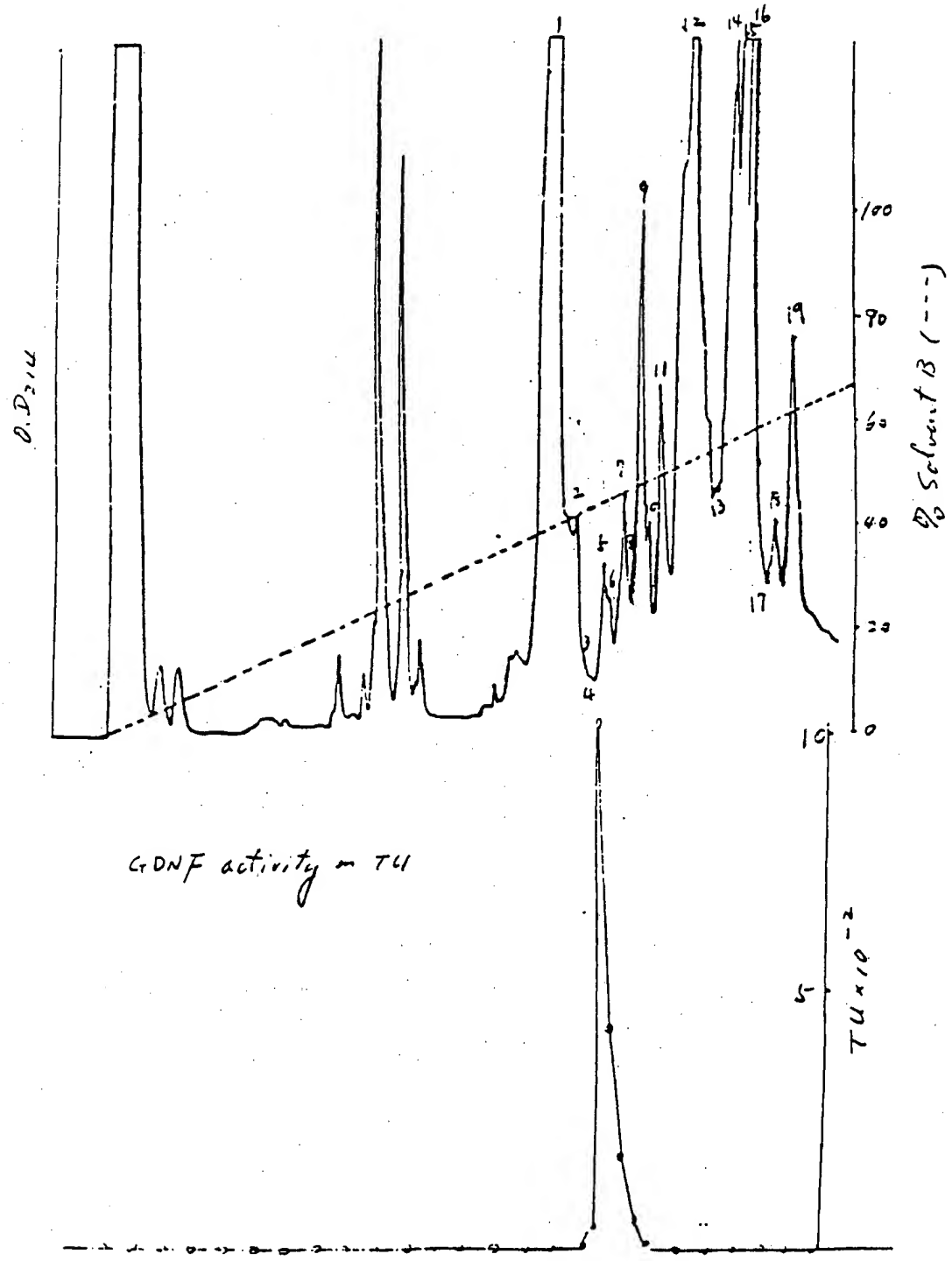
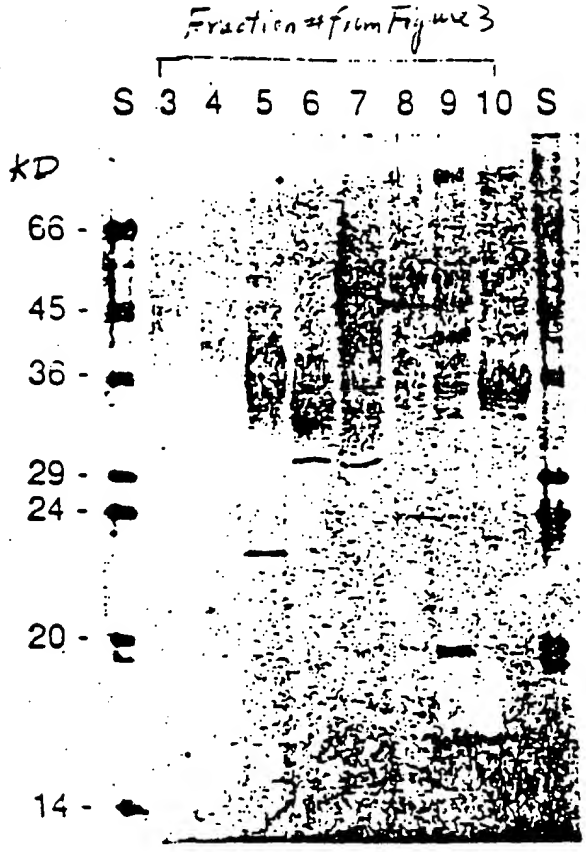
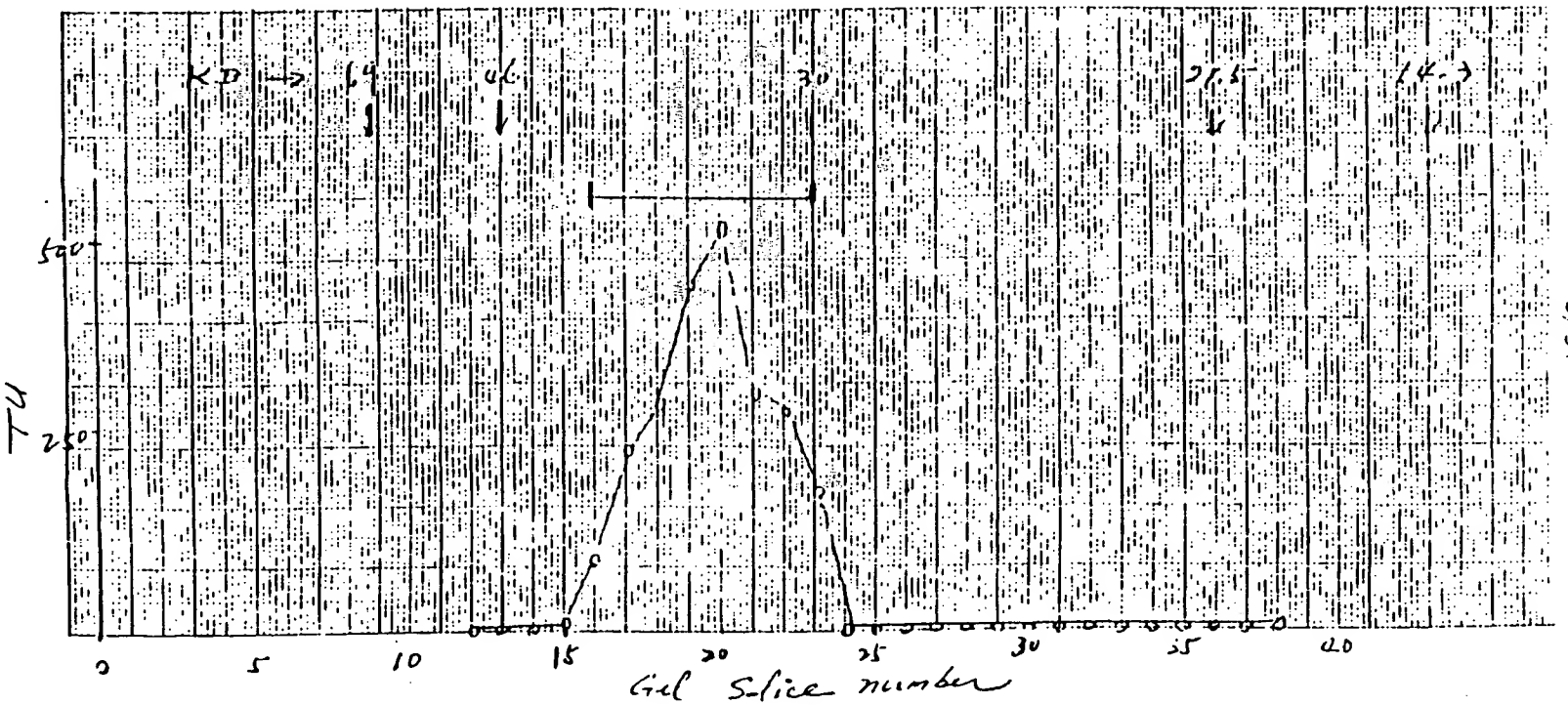

O.D. 280 nm
% Solvent B (---)
100
80
60
40
20
0
10
5
TU x 10⁻²
GDNF activity in TU

Fig. 4. Silver Stained Nonreducing SDS-PAGE



S: Molecular weight Standard

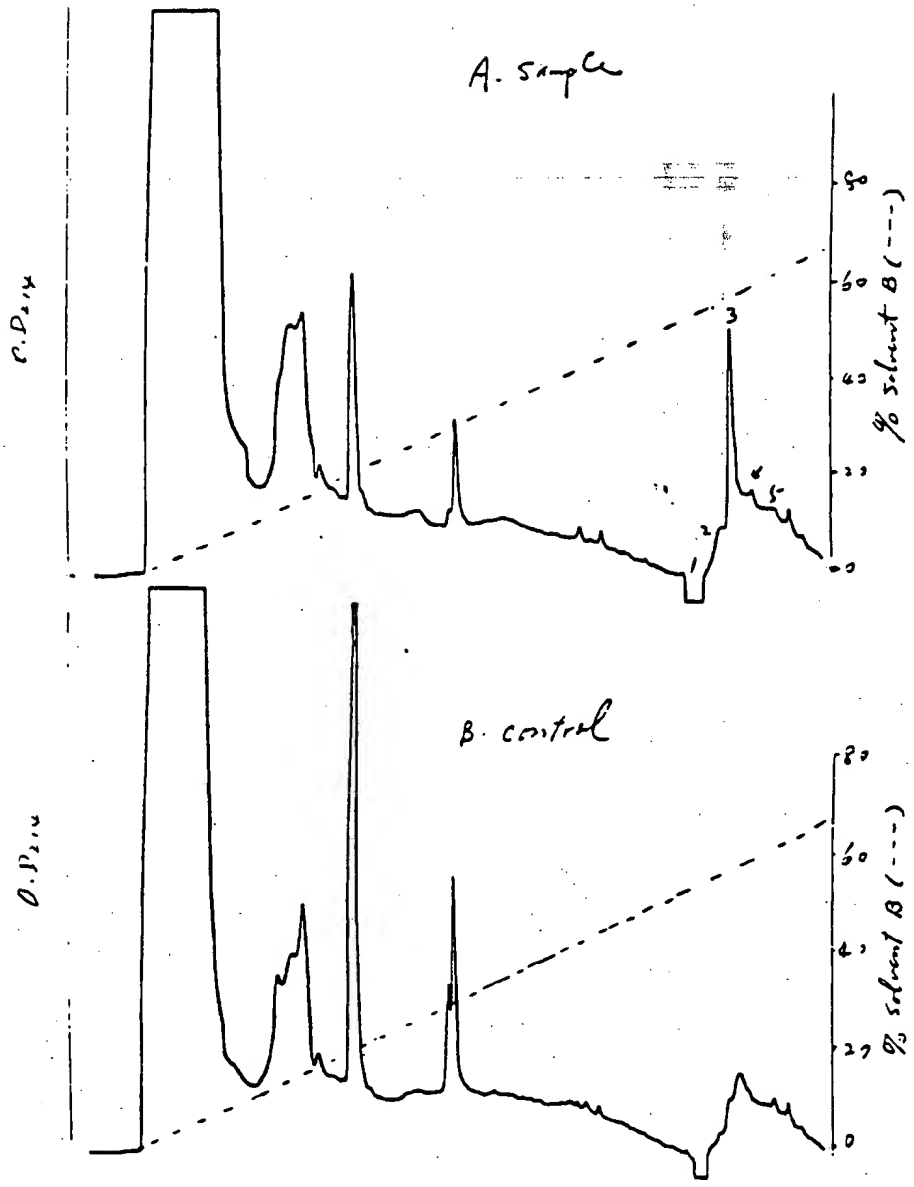
Fig 5. GDNF activity off preparative SDS PAGE



↓ Rainbow protein molecular weight markers (Amersham)

|—| Fractions pooled for further analysis

Fig 6 RP-HPLC of gel slice extract



A. Sample is pooled gel extract from slice #16-23 in Fig 5

B. Control is pooled gel extract from corresponding slices of a blank lane.

Fig. 7 Silver stained non-reducing SDS-PAGE

Lane 1 : sample is from peak 3 in Fig. 6A
S : molecular weight standard

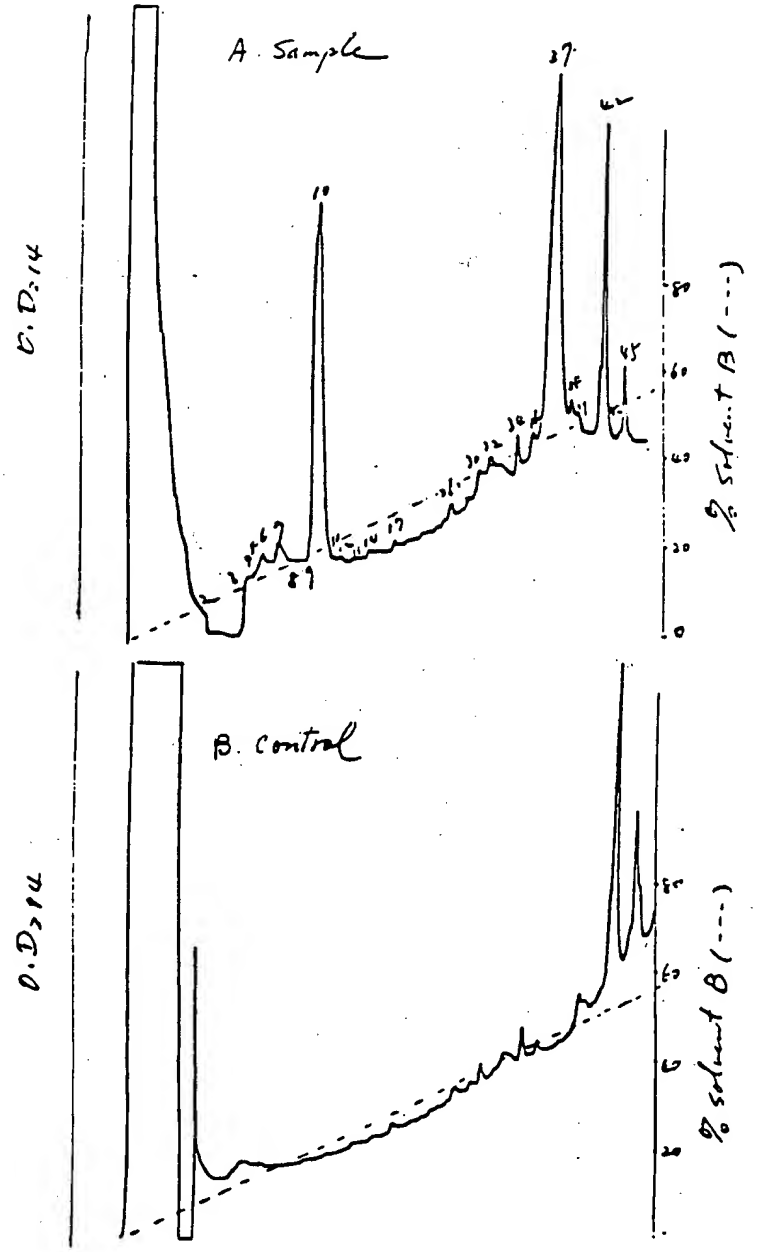
FIGURE 8

Amino-terminal sequence of GDNF

(S)-P-D-K-Q-A-A-A-L-P-R-R-E-(R)-N-( )*-Q-A-A-A-A-(S)-P-(D)-(N)

* no residue could be unequivocally identified in this position
amino acid residues in parenthesis are those identified with less certainty

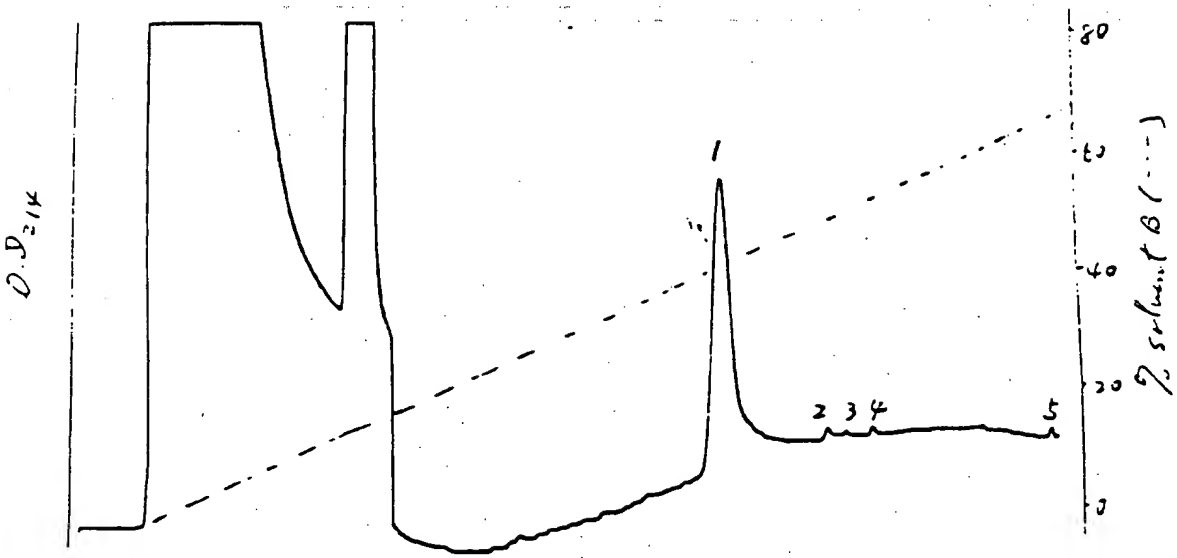
Fig. 7 Rp-HPLC of trypsin-digested GDNF



A. Sample is from fractions 5+6 in Fig. 3

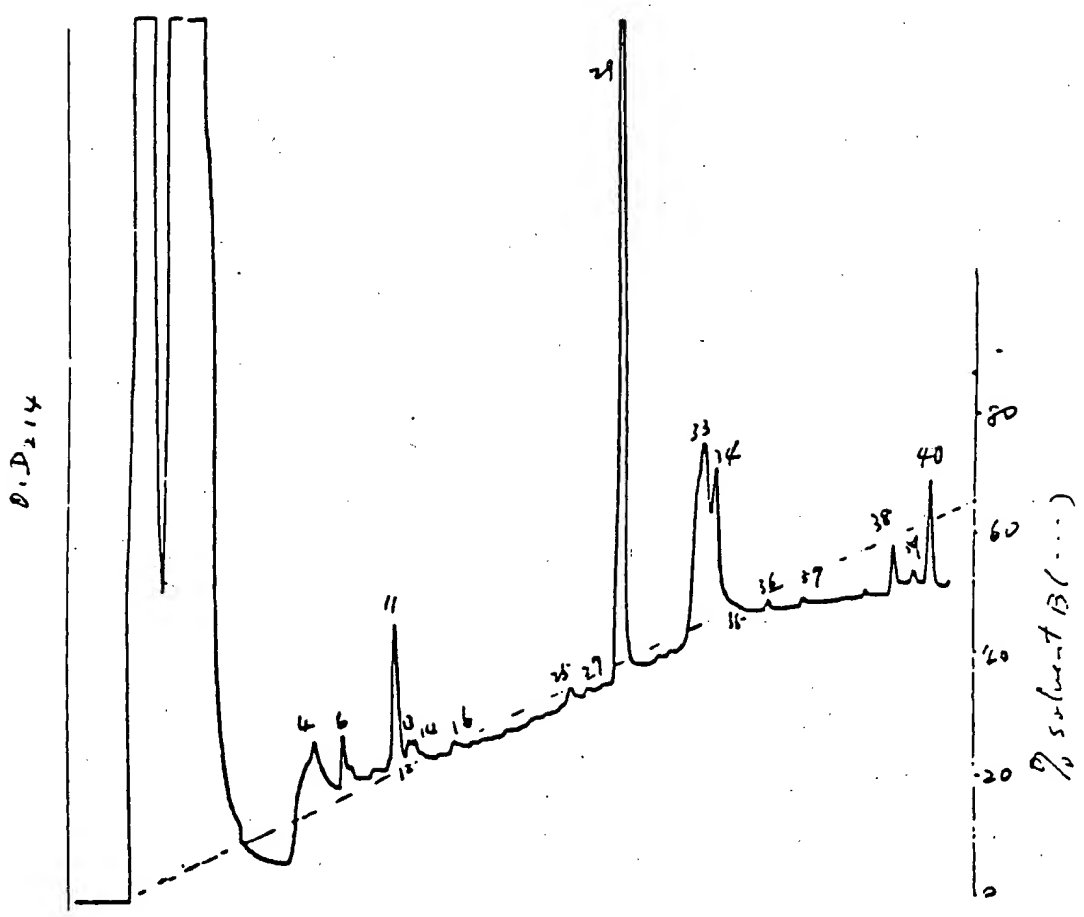
B. A control contains Trypsin only

Fig 10 RP-HPLC of CNBr-treated sample



Sample is from peak 37 in Fig 9A

Fig. 11 RP-HPLC of a reduced CNBr fragment



Sample is from peak 1 in Fig. 10

FIGURE 12

An internal sequence of the GDNF

D-K/Q-I-L-K-N-L-(G)*-(R)-(V)-(R)-(R)-L

*Amino acid residues placed in parentheses are ones identified with less certainty.

FIGURE 13

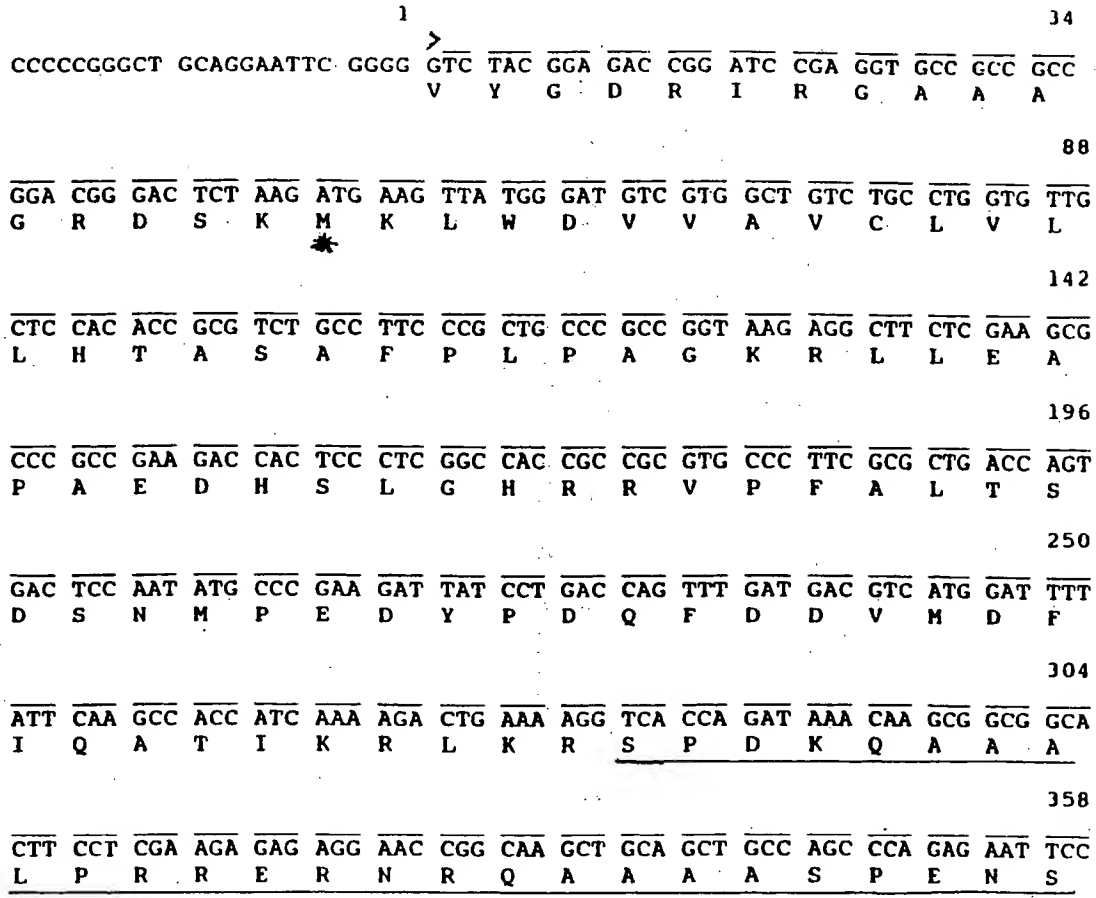

1
34
CCCCCGGGCT GCAGGAATTC GGGG GTC TAC GGA GAC CGG ATC CGA GGT GCC GCC GCC
V Y G D R I R G A A A
88
GGA CGG GAC TCT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC CTG GTG TTG
G R D S K M K L W D V V A V C L V L
142
CTC CAC ACC GCG TCT GCC TTC CCG CTG CCC GCC GGT AAG AGG CTT CTC GAA GCG
L H T A S A F P L P A G K R L L E A
196
CCC GCC GAA GAC CAC TCC CTC GGC CAC CGC CGC GTG CCC TTC GCG CTG ACC AGT
P A E D H S L G H R R V P F A L T S
250
GAC TCC AAT ATG CCC GAA GAT TAT CCT GAC CAG TTT GAT GAC GTC ATG GAT TTT
D S N M P E D Y P D Q F D D V M D F
304
ATT CAA GCC ACC ATC AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA GCG GCG GCA
I Q A T I K R L K R S P D K Q A A A
358
CTT CCT CGA AGA GAG AGG AAC CGG CAA GCT GCA GCT GCC AGC CCA GAG AAT TCC
L P R R E R N R Q A A A S P E N S

FIG 13 CONT

```
                                                                    412
AGA GGG AAA GGT CGC AGA GGC CAG AGG GGC AAA AAT CGG GGG TGC GTC TTA ACT
R   G   K   G   R   R   G   Q   R   G   K   N   R   G   C   V   L   T

                                                                    466
GCA ATA CAC TTA AAT GTC ACT GAC TTG GGT TTG GGC TAC GAA ACC AAG GAG GAA
A   I   H   L   N   V   T   D   L   G   L   G   Y   E   T   K   E   E

                                                                    520
CTG ATC TTT CGA TAT TGT AGC GGT TCC TGT GAA GCG GCC GAG ACA ATG TAC GAC
L   I   F   R   Y   C   S   G   S   C   E   A   A   E   T   M   Y   D

                                                                    574
AAA ATA CTA AAA AAT CTG TCT CGA AGT AGA AGG CTA ACA AGT GAC AAG GTA GGC
K   I   L   K   N   L   S   R   S   R   R   L   T   S   D   K   V   G

                                                                    628
CAG GCA TGT TGC AGG CCG GTC GCC TTC GAC GAC GAC CTG TCG TTT TTA GAC GAC
Q   A   C   C   R   P   V   A   F   D   D   D   L   S   F   L   D   D

                                                                    682
AGC CTG GTT TAC CAT ATC CTA AGA AAG CAT TCC GCT AAA CGG TGT GGA TGT ATC
S   L   V   Y   H   I   L   R   K   H   S   A   K   R   C   G   C   I

                                                                    745
TGA CCCTGGCTCC AGAGACTGCT GTGTATTGCA TTCCTGCTAC AGTGCGAAGA AAGGGACCAA
```

FIG. 13 CONT.

815

GGTTCCCAGG AAATATTTGC CCAGAAAGGA AGATAAGGAC CAAGAAGGCA GAGGCAGAGG CGGAAGAAGA

875

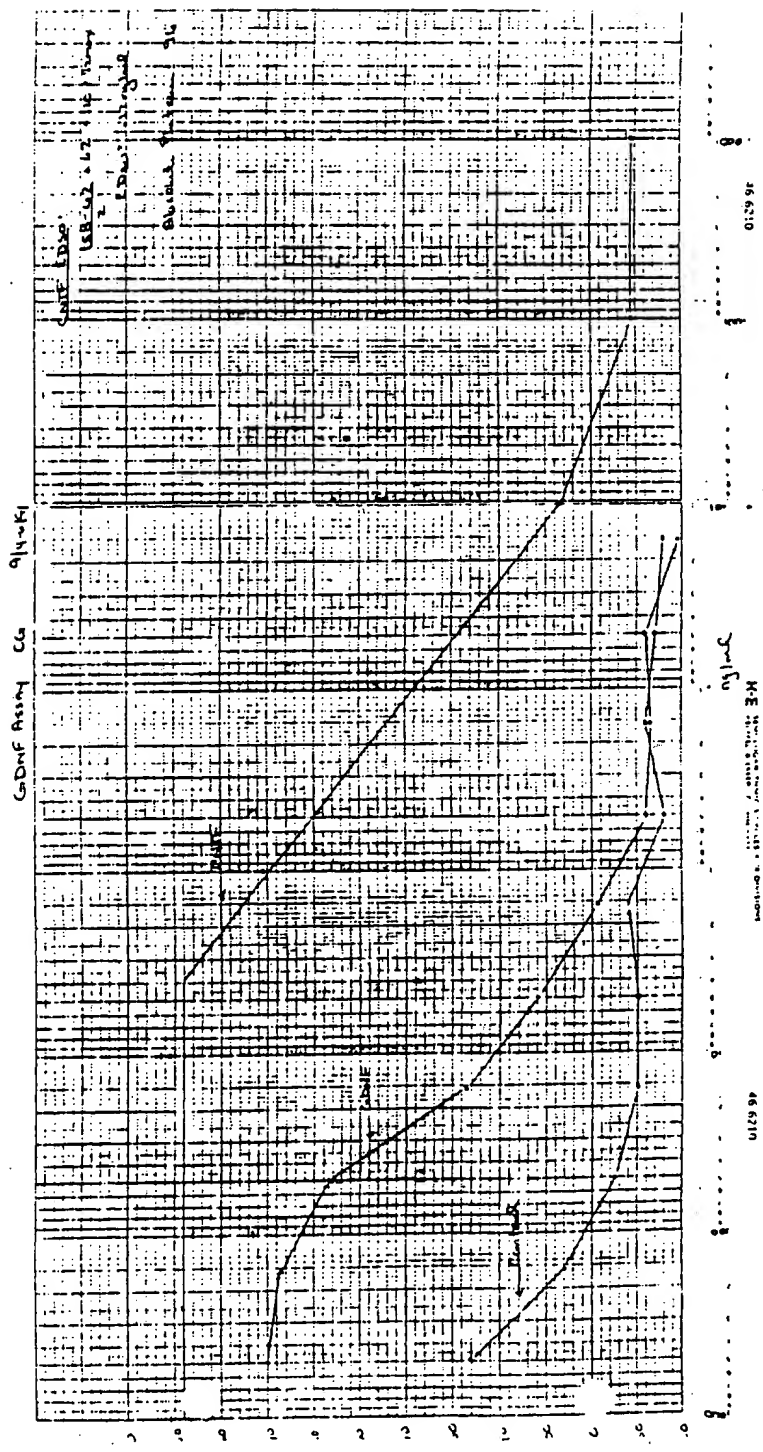
AGAAGAAAAG AAGGACGAAG GCAGCCATCT GTGGGAGCCT GTAGAAGGAG GCCCAGCTAC AG

FIGURE 14

S	P	D	K	Q	A	A	A										
L	P	R	R	E	R	N	R	Q	A	A	A	A	S	P	E	N	S
R	G	K	G	R	R	G	Q	R	G	K	N	R	G	C	V	L	T
A	I	H	L	N	V	T	D	L	G	L	G	Y	E	T	K	E	E
L	I	F	R	Y	C	S	G	S	C	E	A	A	E	T	M	Y	D
K	I	L	K	N	L	S	R	S	R	R	L	T	S	D	K	V	G
Q	A	C	C	R	P	V	A	F	D	D	D	L	S	F	L	D	D
S	L	V	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I

BJS:tec:4:September 9, 1991 synergen\gdnf.2

FIGURE 15



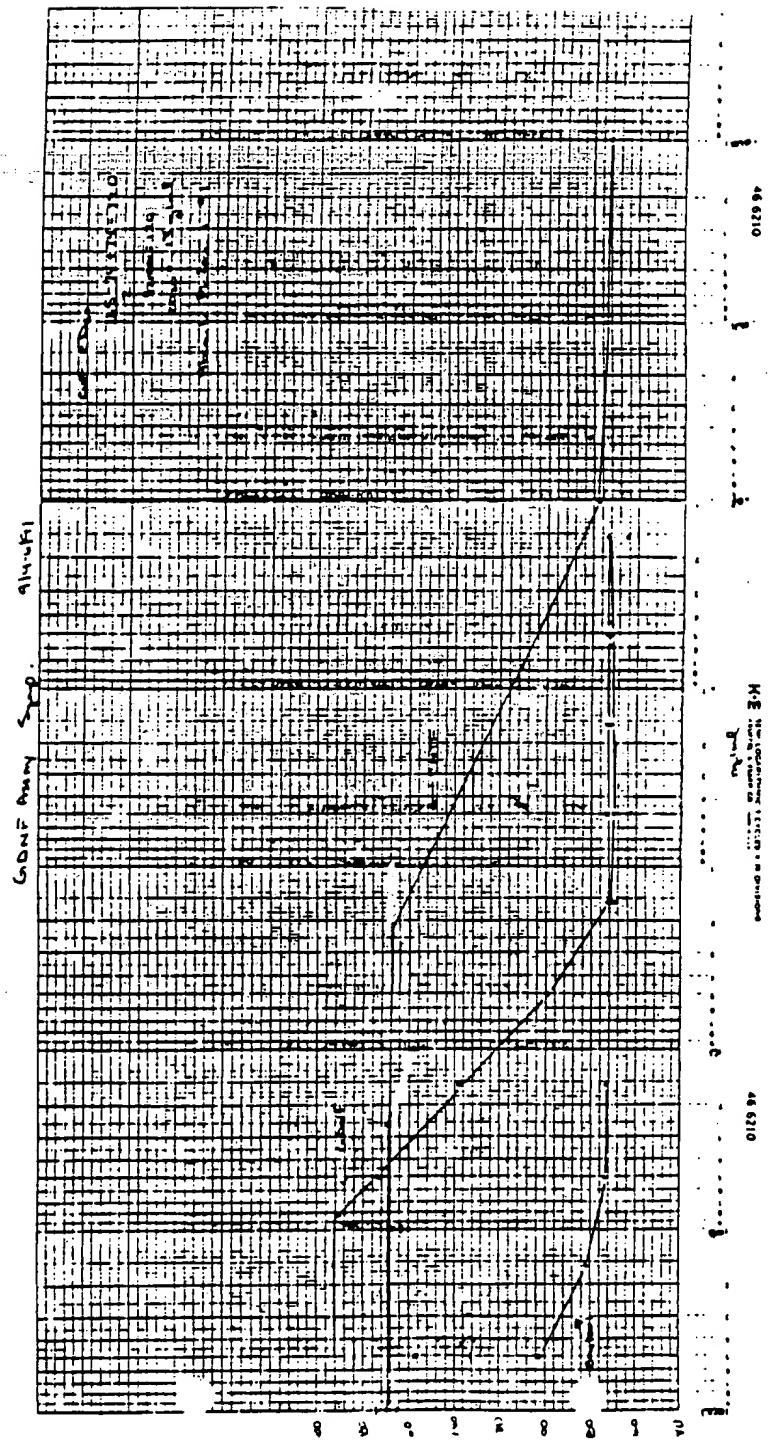
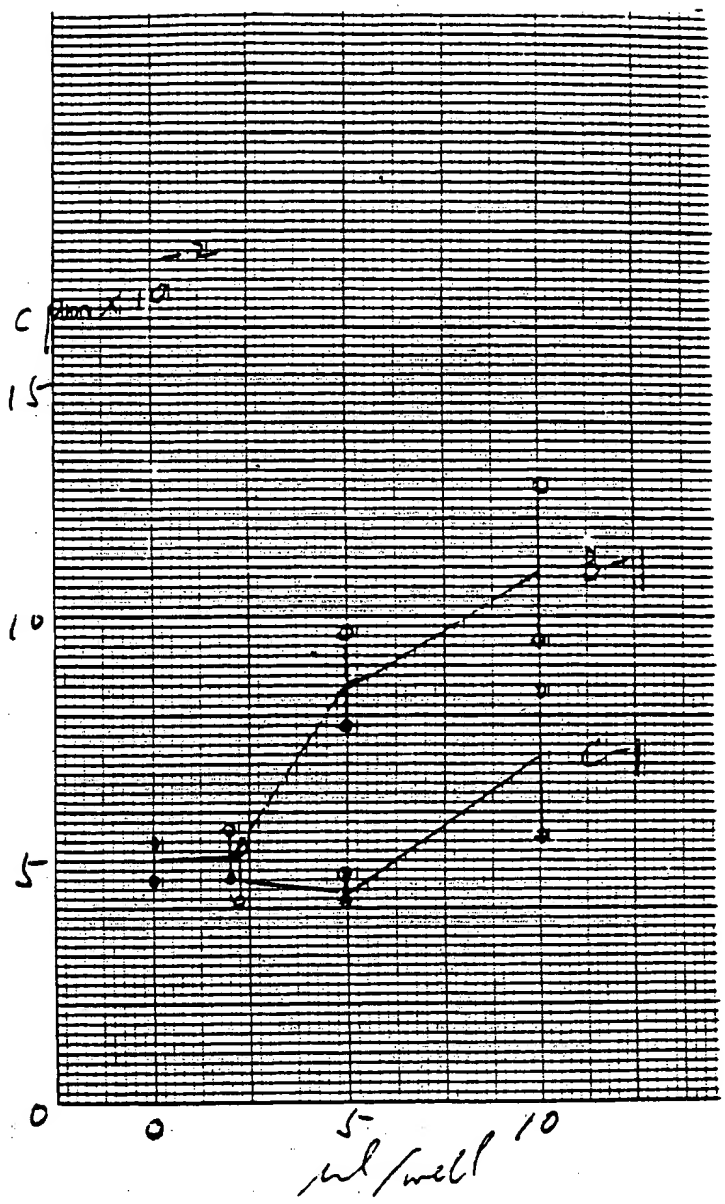


FIGURE 16

Figure 19/31 17



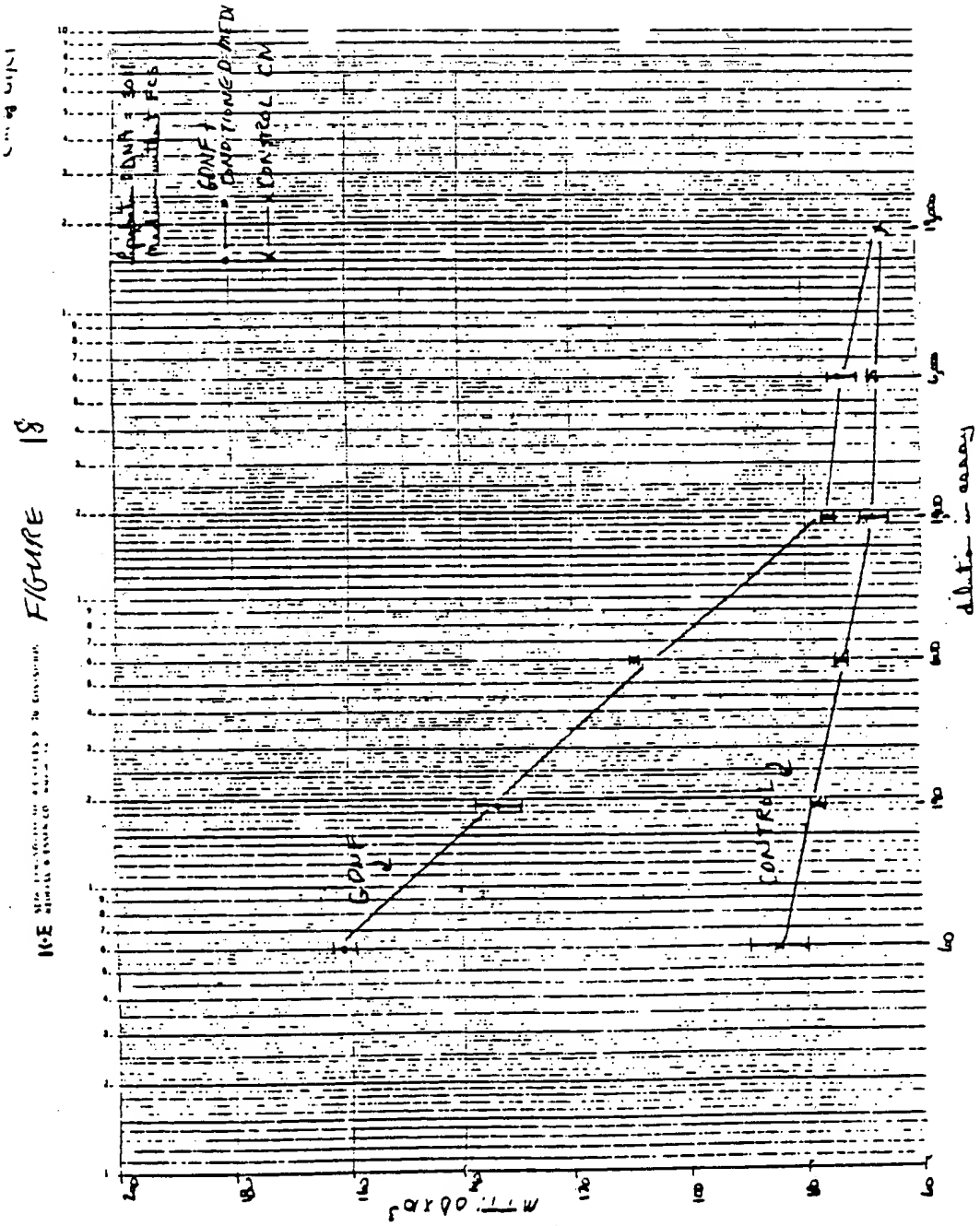


FIGURE 15
GOAT
CONTROL
GOAT
CONTROL CM
days post-challenge
mean body temperature

FIGURE 19

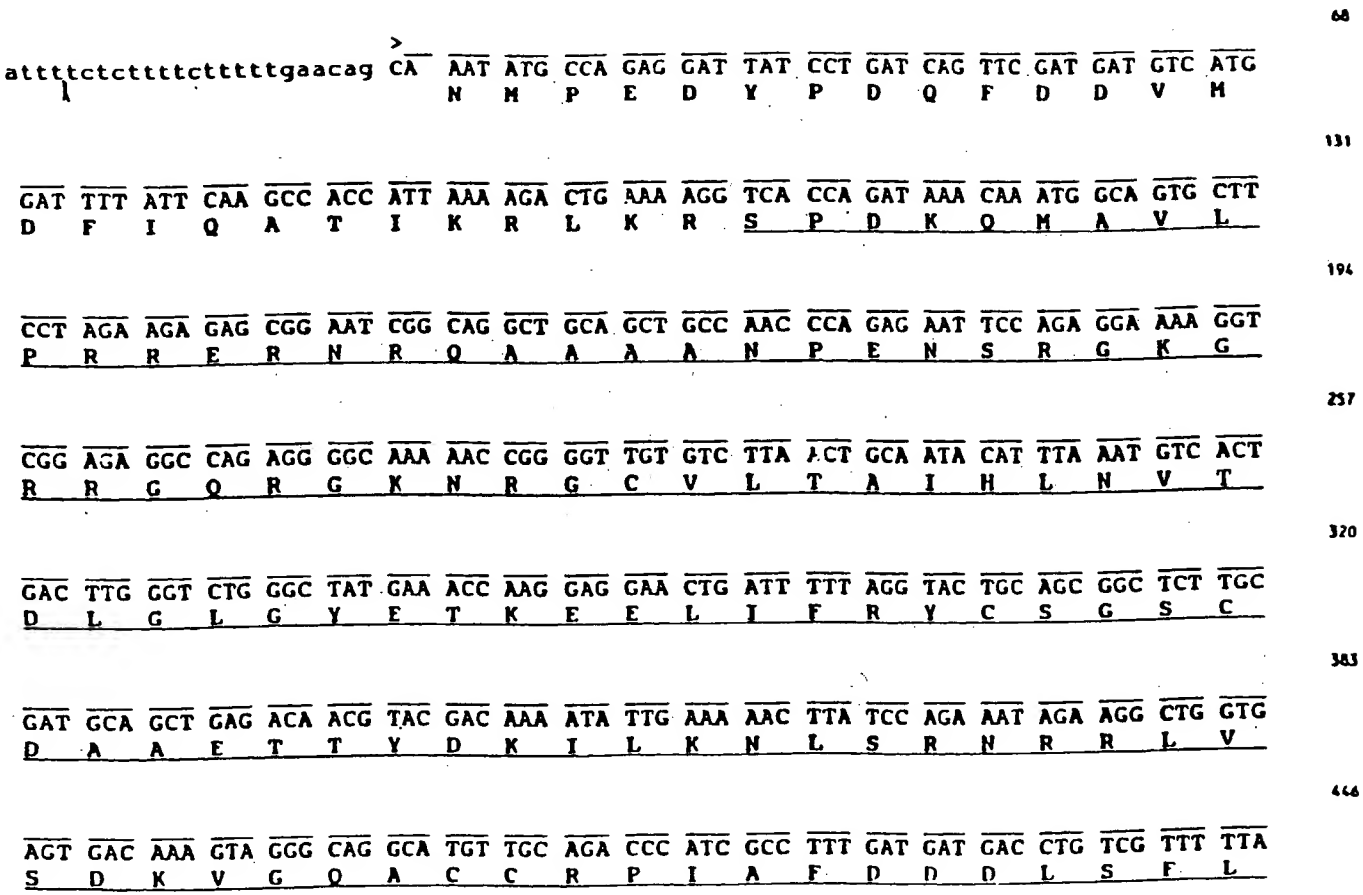

1
68
atttctctttttctttttgaacag CA AAT ATG CCA GAG GAT TAT CCT GAT CAG TTC GAT GAT GTC ATG
N M P E D Y P D Q F D D V M
131
GAT TTT ATT CAA GCC ACC ATT AAA AGA CTG AAA AGG TCA CCA GAT AAA CAA ATG GCA GTG CTT
D F I Q A T I K R L K R S P D K Q M A V L
194
CCT AGA AGA GAG CGG AAT CGG CAG GCT GCA GCT GCC AAC CCA GAG AAT TCC AGA GGA AAA GGT
P R R E R N R Q A A A A N P E N S R G K G
257
CGG AGA GGC CAG AGG GGC AAA AAC CGG GGT TGT GTC TTA ACT GCA ATA CAT TTA AAT GTC ACT
R R G Q R G K N R G C V L T A I H L N V T
320
GAC TTG GGT CTG GGC TAT GAA ACC AAG GAG GAA CTG ATT TTT AGG TAC TGC AGC GGC TCT TGC
D L G L G Y E T K E E L I F R Y C S G S C
383
GAT GCA GCT GAG ACA ACG TAC GAC AAA ATA TTG AAA AAC TTA TCC AGA AAT AGA AGG CTG GTG
D A A E T T Y D K I L K N L S R N R R L V
446
AGT GAC AAA GTA GGG CAG GCA TGT TGC AGA CCC ATC GCC TTT GAT GAT GAC CTG TCG TTT TTA
S D K V G Q A C C R P I A F D D D L S F L

FIG. 19 CONT.

```
GAT GAT AAC CTG GTT TAC CAT ATT CTA AGA AAG CAT TCC GCT AAA AGG TGT GGA TGT ATC TGA
D   D   N   L   V   Y   H   I   L   R   K   H   S   A   K   R   C   G   C   I   .
```

> ctccggctccagagactgctgtgtattgcattcctgctacagtgcaaagaaag

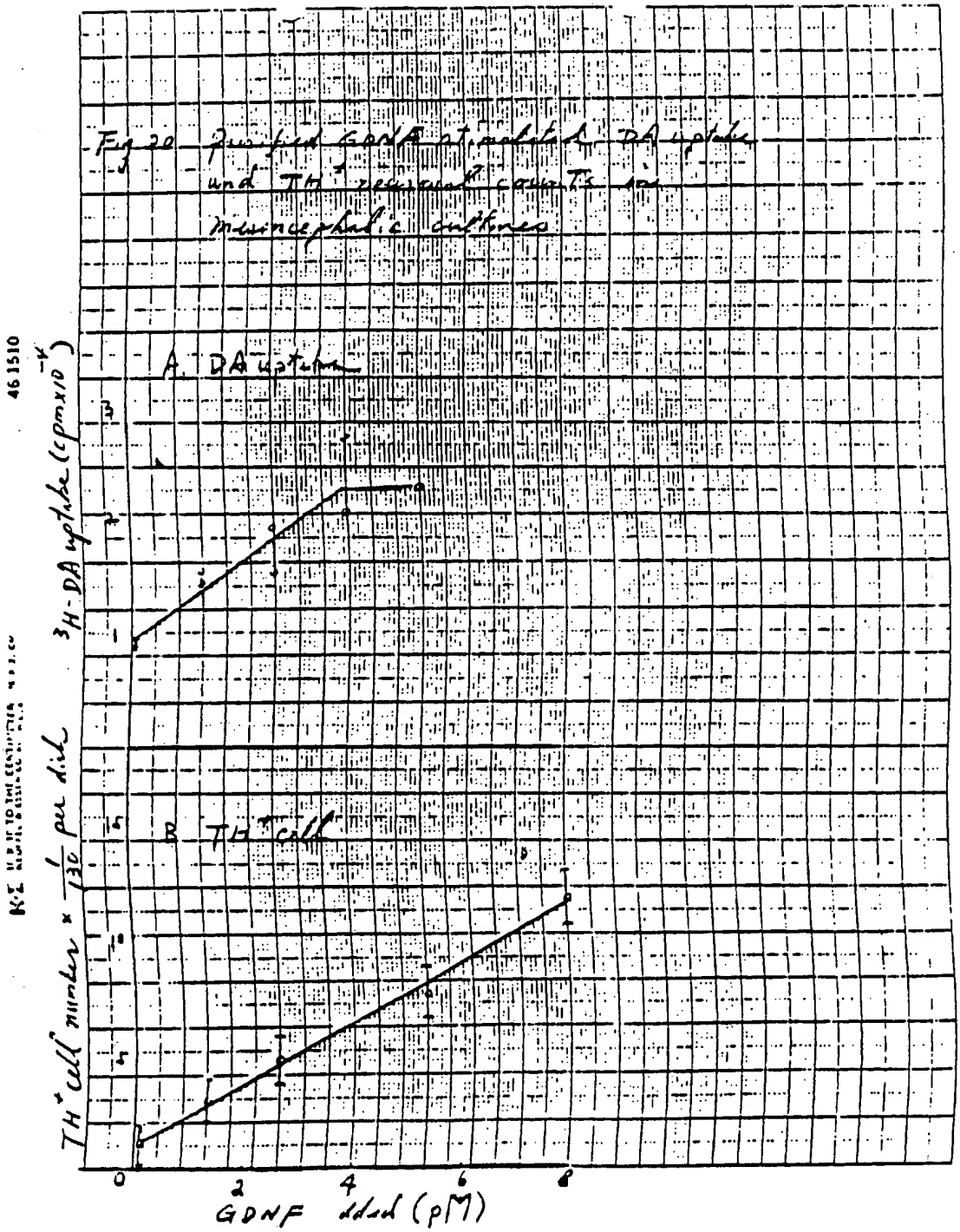


Fig 20 Purified GDNF stimulated DA uptake and TH+ neuronal counts in mesencephalic cultures
A. DA uptake
B. TH+ cell
^{3}H-DA uptake (cpm x 10^{-2})
TH+ cell number x $\frac{1}{130}$ per dish
GDNF added (pM)
0
2
4
6
8

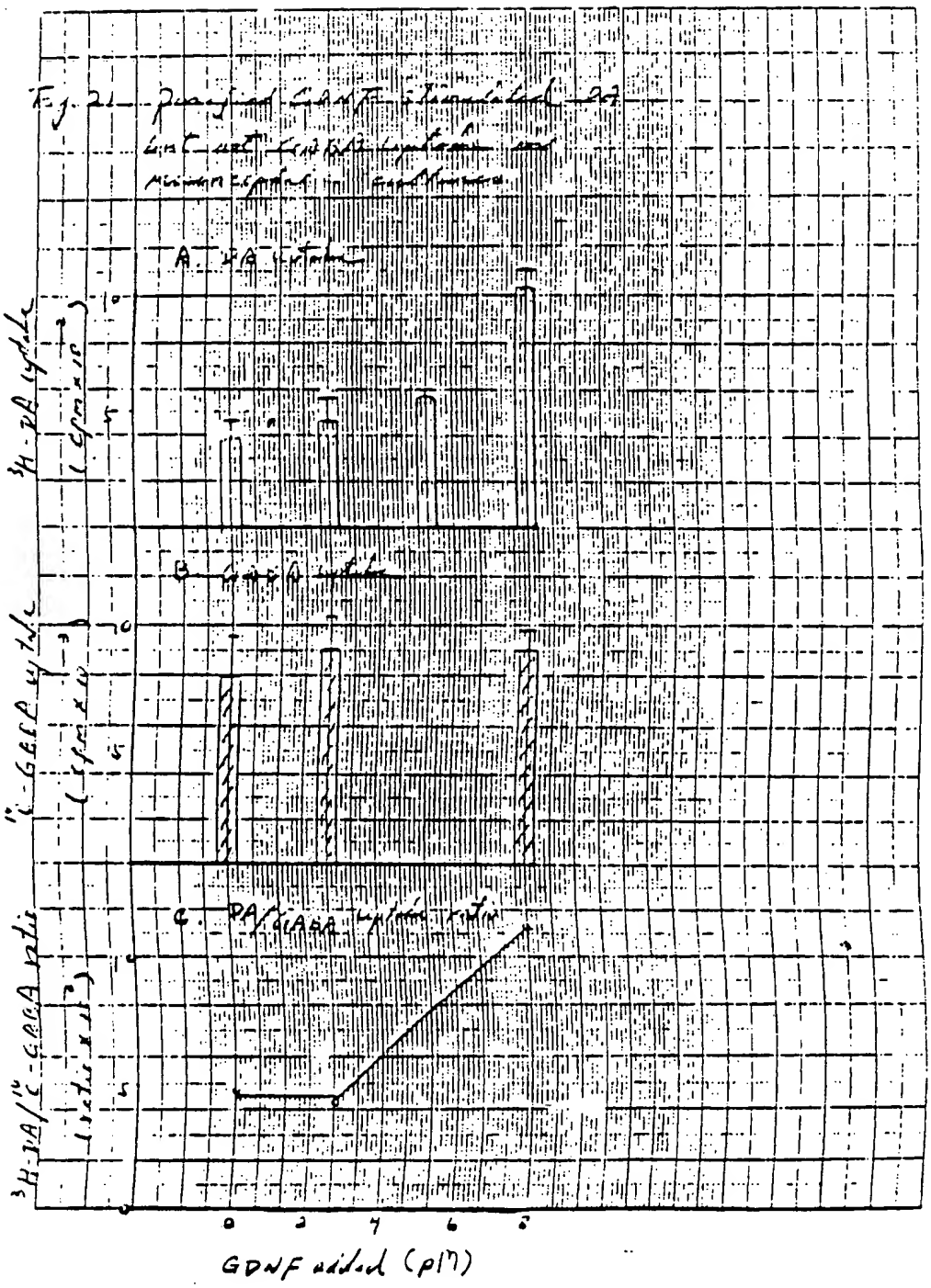

GDNF added (pM)
0
2
4
6
8

Figure 22

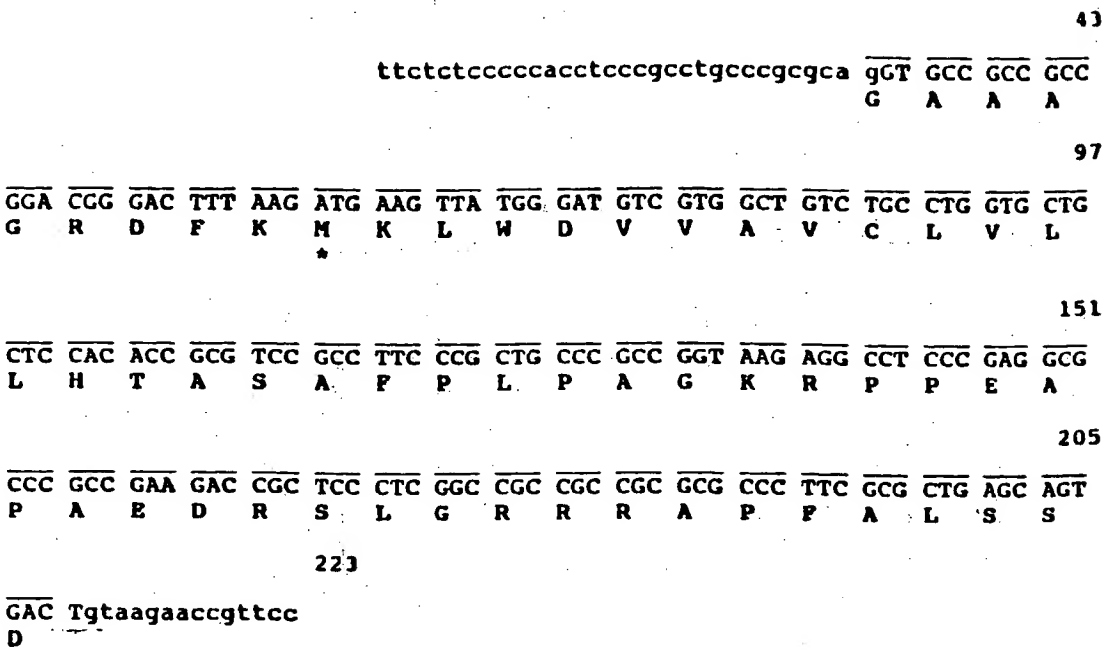
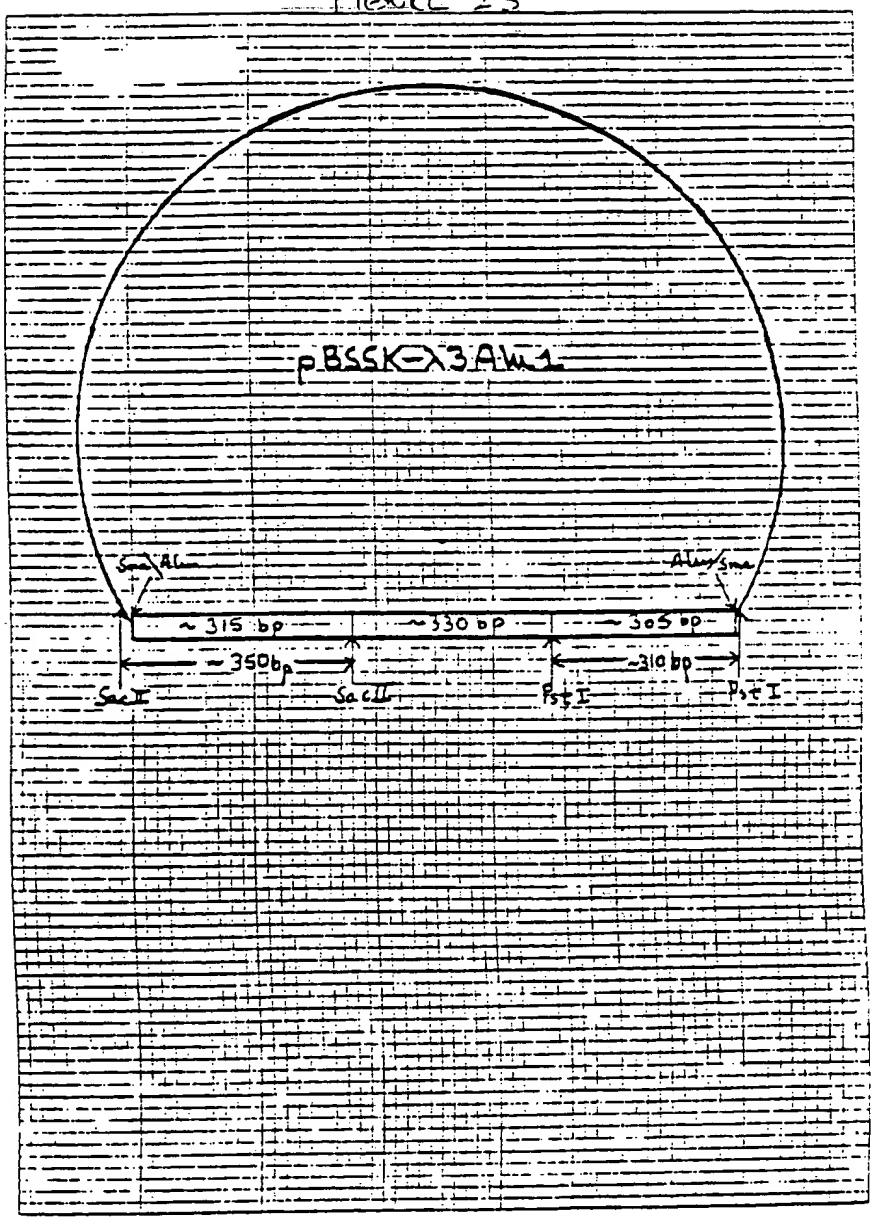

43
ttctctcccccacctcccgcctgcccgcgca gGT GCC GCC GCC
G A A A
97
GGA CGG GAC TTT AAG ATG AAG TTA TGG GAT GTC GTG GCT GTC TGC CTG GTG CTG
G R D F K M K L W D V V A V C L V L
*
151
CTC CAC ACC GCG TCC GCC TTC CCG CTG CCC GCC GGT AAG AGG CCT CCC GAG GCG
L H T A S A F P L P A G K R P P E A
205
CCC GCC GAA GAC CGC TCC CTC GGC CGC CGC CGC GCG CCC TTC GCG CTG AGC AGT
P A E D R S L G R R R A P F A L S S
223
GAC Tgtaagaaccgttcc
D

FIGURE 23


pBSSK-λ3AM1
~315 bp
~330 bp
~305 bp
~350bp
~310 bp
SacI
SacII
PstI
PstI

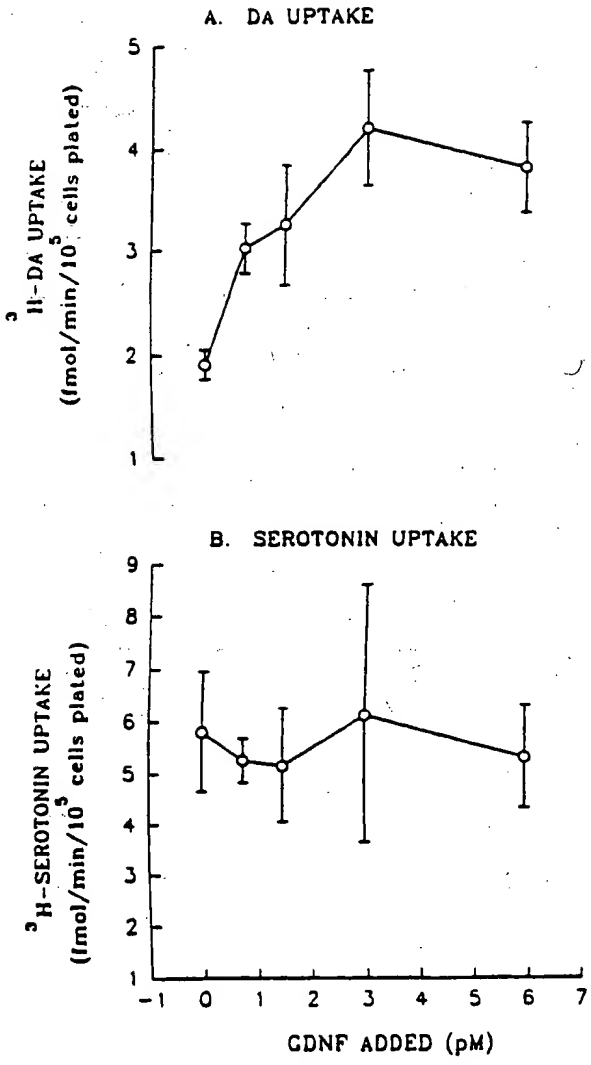

A. DA UPTAKE
^{3}H-DA UPTAKE
(fmol/min/10^5 cells plated)
5
4
3
2
1
B. SEROTONIN UPTAKE
^{3}H-SEROTONIN UPTAKE
(fmol/min/10^5 cells plated)
9
8
7
6
5
4
3
2
1
−1
0
1
2
3
4
5
6
7
GDNF ADDED (pM)

FIGURE 24

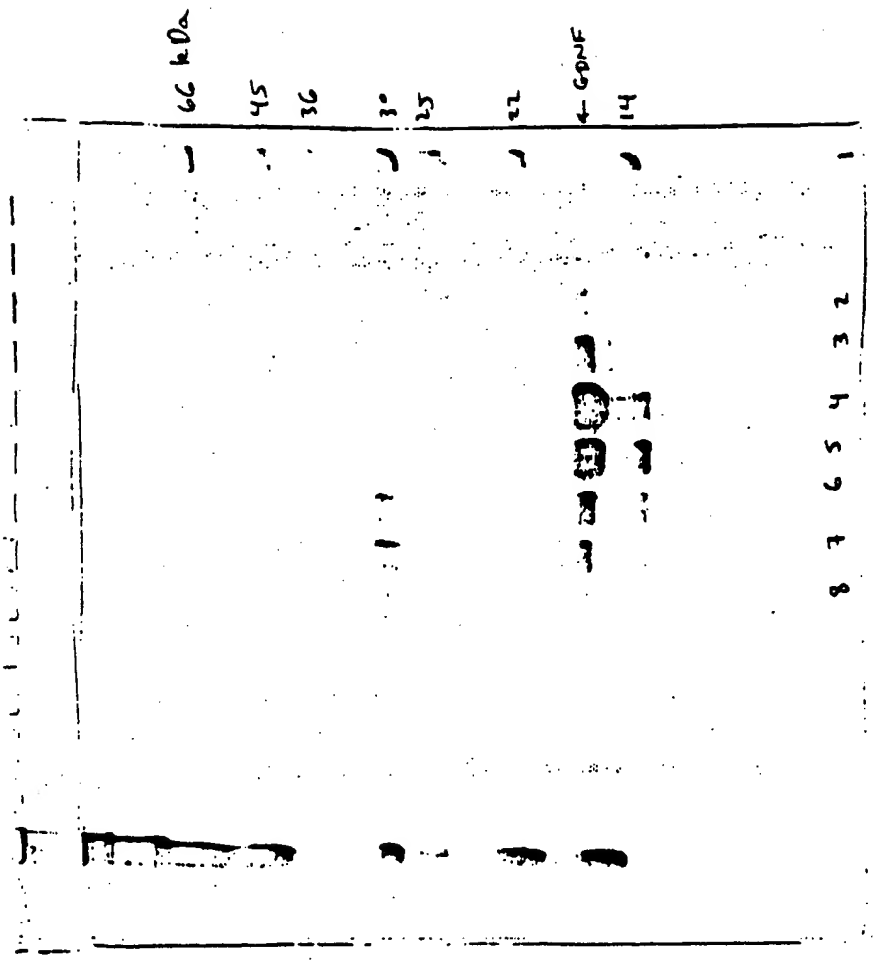


FIGURE 25

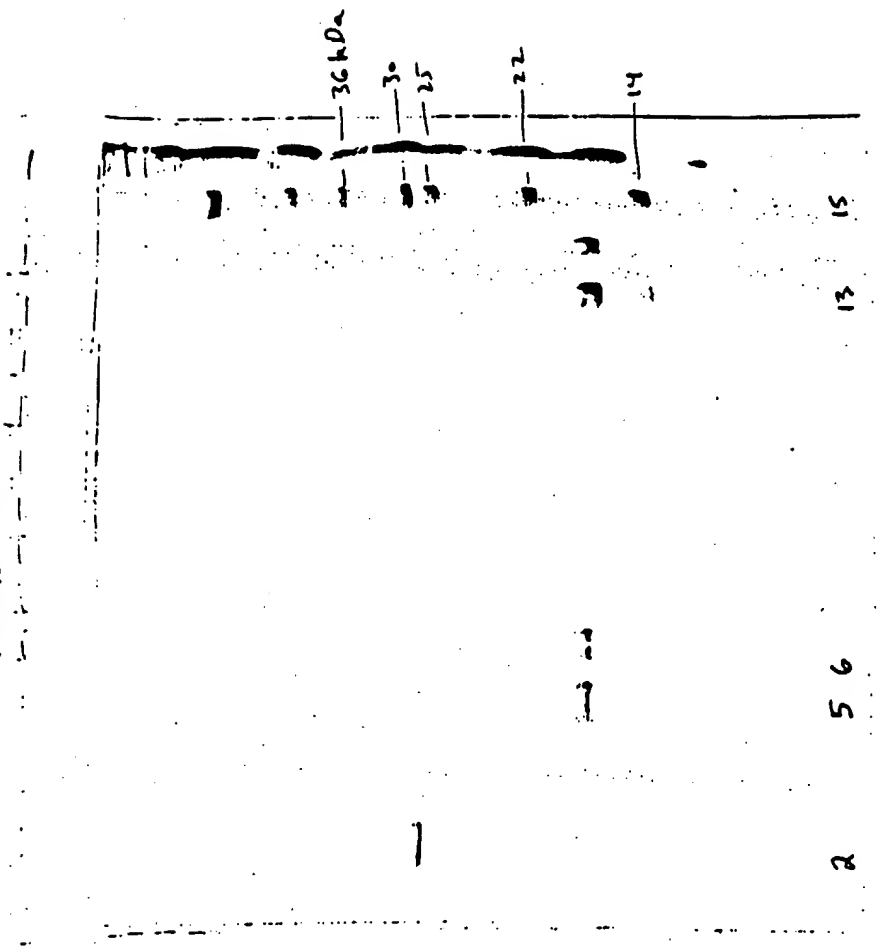


FIGURE 26

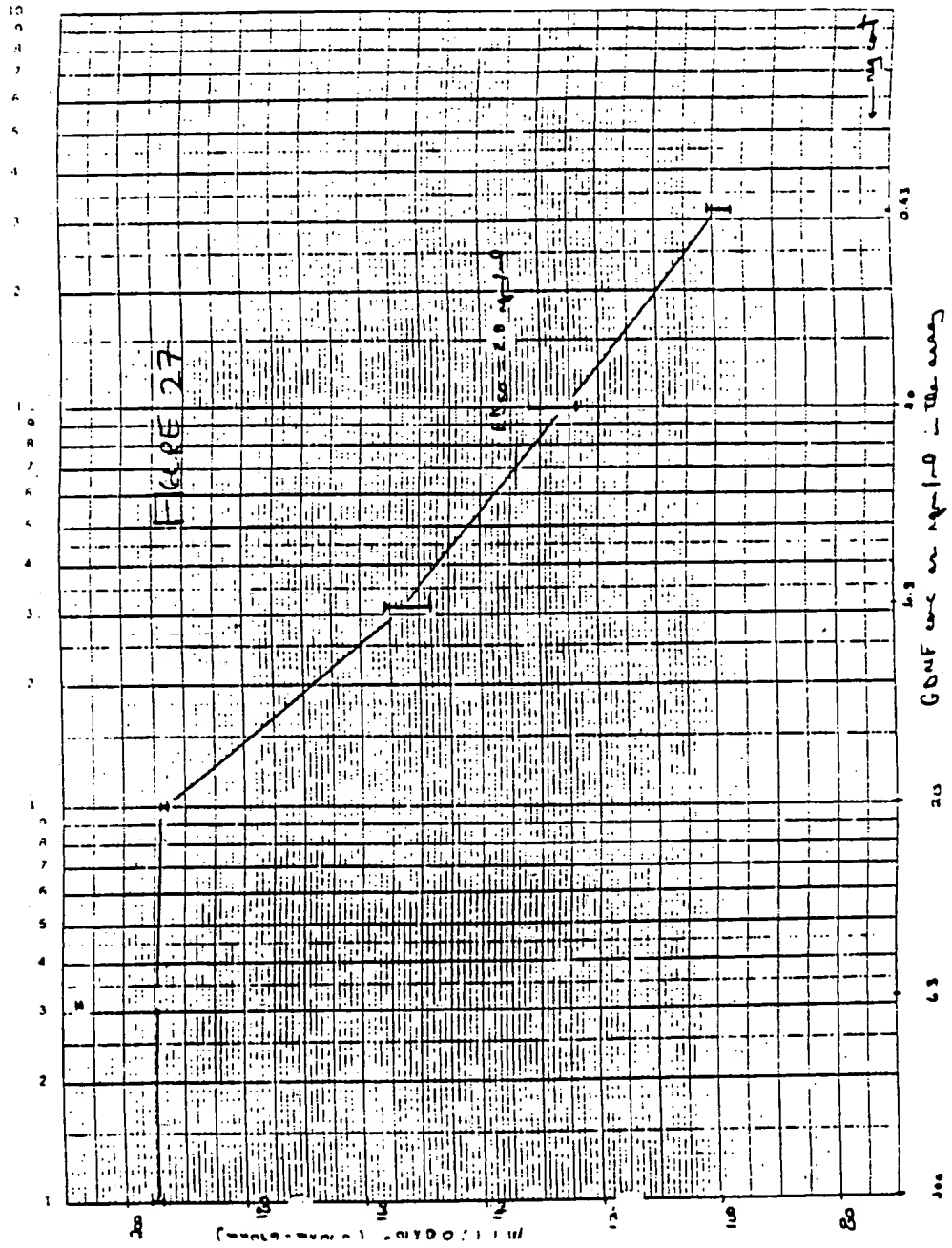


FIGURE 27

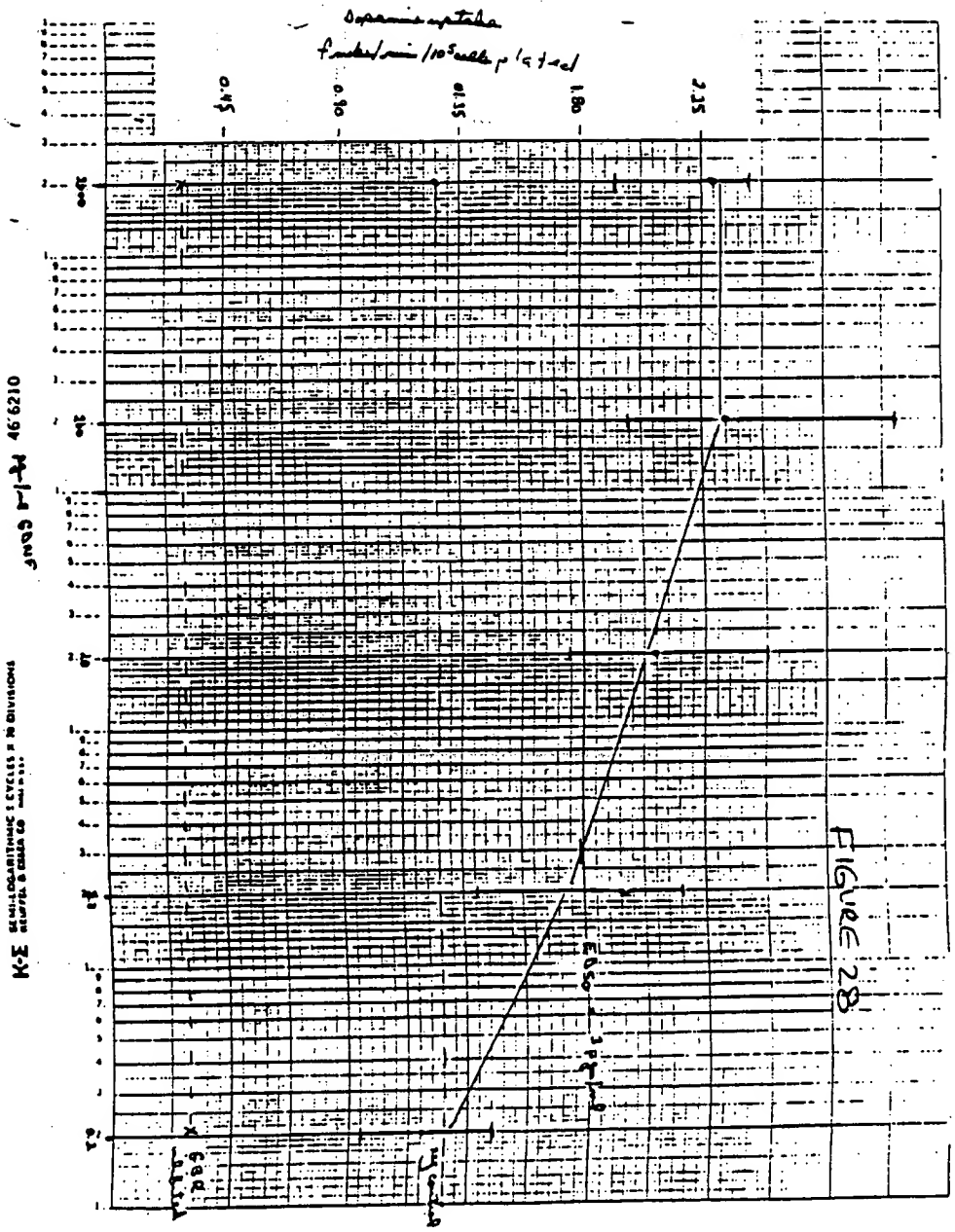

0.45
0.90
1.35
1.80
2.25
K-E SEMI-LOGARITHMIC 2 CYCLES X 70 DIVISIONS
46 6210

FIGURE 28